Chapter 24

Bovine Babesiosis: The Role of Vaccination in Cattle Protection

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ABSTRACT

Babesiosis causes a significant loss in livestock productivity especially in tropical and subtropical areas. Various species of protozoan parasites cause the disease belonging to the genus *Babesia*, which are intraerythrocytic pathogen. Bovine Babesiosis has a major impact on the cattle industry through disease detection, prevention, treatment and vector control costs. *Babesia bovis* causes severe infection that characterized by anorexia, hemoglobinuria, fever, hemolytic anemia, and frequently death. Vaccines play a vital role in preventing those infections where treatment is limited or difficult, also for controlling diseases, especially in prevalent areas. Vaccine has a prolog history in protection effects against pathogens, by vaccination the disease burden can be reduced and the health status of animals can be maintained. Babesiosis prophylaxis has been achieved through the widespread use of live attenuated *Babesia* vaccine for calves since the mid-sixties. An achievement in veterinary vaccination successfully allowed the eradication of some devastating diseases. Challenges for developing and innovating in vaccine against new strains of various pathogens are continued. Recently vaccines are available for various parasitic infection in different hosts including bovine babesiosis, which they act on activation of host immune system, and reducing the effects of special pathogen through providing the protective response, some of them are lifelong. The lack of effective medications and the diversity of causative agents makes it difficult to control bovine babesiosis, however, various procedures were followed for developing of effective vaccine against *Babesia* species in cattle from different endemic regions globally.

KEYWORDS
Babesiosis, Vaccination, Immunization, *Babesia*, Cattle

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INTRODUCTION

Babesiosis is a parasitic disease with great significance, caused by protozoan parasites belonging to the genus *Babesia* and transmitted by ticks (Hunfeld et al., 2008). They are obligate intra-erythrocytic parasites (Westblade et al., 2017).

Victor Babes was the first who discover *Babesia* parasites in 1888 while investigating cattle herds detected with hemoglobinuria and initially named them *Haematococcus bovis* (Babes, 1888), while he didn't notice the presence of ticks in diseased cattle, but in 1893, Theobald Smith and Frederick Kilborne from United States demonstrating that *Boophilus annulatus* tick was responsible for transmitting of the disease and termed the tick fever (Smith and Kilborne, 1893).

There are around 100 species of *Babesia (B.)* known to exist in mammals (Schuster, 2002). Cattle are the main host and reservoirs for four species *B. bovis, B. bigemina, B. divergens*, and *B. major* (Iseki et al., 2010). In the tropics and subtropics, *Babesia bovis, B. bigemina, and B. divergens* are known to induce severe clinical bovine babesiosis (Bock et al., 2004). The disease is endemic in tropical and temperate regions of the world, where an estimated 500 million cattle are at danger of being infected (He et al., 2021). The infection initiates when parasitic sporozoites that are secreted from the salivary glands of ticks invade RBCs and then grow, multiply, and exit. This cycle continues when newly formed RBCs are invaded by the egressed merozoites. It is anticipated that stage-specific gene expression in the parasite will be the cause of these modifications (Elsworth and Duraisingh, 2020). When it subverts the hosts' immune systems, resulting in both transient and long-term infections (Alzan et al., 2022).

The most severe form of the disease is caused by *B. bovis* (Brown and Palmer, 1999). The acute babesiosis in bovine is characterized by fever (> 40°C), varying degrees of anemia, and hemolysis. Clinical indications of anemia can appear suddenly and include pale mucous membranes, inappetence, decreased milk production, weakness, lethargy, and elevated heart and respiratory rates (Shkap et al., 2007). The disease's acute phase may result in death or a lifelong infection. Adhesion of infected red blood cells in the capillaries of the brain, liver, and lungs, among other essential organs, is another sign of acute *B. bovis* infection (Sondgeroth et al., 2014).

Prompt diagnosis and anti-babesial agent treatment are essential for recovery, delays in therapy may cause the infected animals to pass away (Sivakumar et al., 2018). The mortality rate due to *B. bovis* infection is high, particularly in susceptible breeds or animals that have never been infected, however, species like *Bos indicus* cattle, which are native to *Babesia* endemic regions, exhibit mild to moderate clinical symptoms as a result of their innate resistance to the illness (Bock et al., 2004).

Babesiosis may result in either death or persistent infection in recovered animals from the acute phase. Animals with persistent infection serve as reservoirs for competent tick transmission (He et al., 2021). Detection of infected carrier cattle with *Babesia* parasites is crucial for risk evaluation since uninfected cattle can contract the parasites from ticks (Alvarez et al., 2019). A comprehensive calculation of the enormous expenses associated with babesiosis has taken into account mortality, abortions, lost milk and meat output, and control methods (Reyes-Sandoval et al., 2016).

In endemic areas, prevention of babesiosis in bovine is rely on the usage of acaricides for controlling of ticks, and live attenuated vaccines. Animals that have received vaccinations or have recovered from infections acquire adaptive immunity, but they still harbor the infection and could act as carriers of the disease (Hakimi and Verocai, 2023).

Administration of anti-babesial drugs ensuing the residues of chemicals in milk and meat which accompany harmful effects on public health. Furthermore, the use of acaricidal and anti-babesial medications at random doses leads to the development of resistance to the causing organisms and vectors. Therefore, strengthening immunization programs is crucial, especially in areas where a large number of animals are at risk (Maqbool et al., 2018).

The innate immune response in the spleen is responsible for clearing naive animals from acute babesiosis produced by *B. bovis*, while protection of continuously infected cattle from clinical illness (concomitant immunity) or vaccinated cattle depend on rapid activation of memory and effector CD4+ T cells for IFN-γ secretion and supporting in protective antibodies production (Brown et al., 2006a).

Recent sight about adaptive immune response against *B. bovis* involves a various response from both T and cells which characterized by the activation of CD4+ Th1-lymphocytes, and the production of neutralizing antibodies directed against parasite surface antigens at the erythrocyte membrane and extracellular merozoites. In addition, IFN-γ is necessary to stimulate macrophages to generate babesiacidal molecules and to enhance the opsonizing IgG2 antibody response (Brown et al., 2006a).

Applying live, attenuated organisms as immunogens has allowed for the development of solid immunity subsequent to infection (Maqbool et al., 2018). Consequently, for countries where the disease is enzootic, the development of dependable, safer, and equally protective vaccine against bovine babesiosis would be extremely helpful (Ortiz et al., 2019).

Bovine Babesiosis

After trypanosomiasis, babesiosis is the second most frequent disease transmitted to animals by ticks (Yabsley and Shock, 2013). *Babesia* species have an impact in tropical and subtropical regions where Ixodidae ticks are prevalent, however *B. bovis, B. bigemina* and *B. divergens* are more prevalent having a significant influence on the cattle sector (Bock et al., 2004). Bovine babesiosis commonly caused by *Babesia bovis*. It can be found in Asia, Africa, Australia, portions of Southern Europe, Central, South, and Southern North America. One of its main vectors is *Rhipicephalus microplus*, via transovarial transmission, infected female ticks transmit the infection to their offspring (Hakimi and Verocai, 2023).

Babesia species have a complicated life cycle that includes sexual reproduction in the gut of the definitive tick vectors, and asexual reproduction when the parasite is present inside the erythrocytes of its mammalian hosts. The parasite can also change into other distinct stages during its development in the tick's body, such as gametes, kinetes, and sporozoites. It can also invade various tissues, such as the tick progeny's ovary and egg tissues, which are essential steps for the parasite to spread transovarially (Alzan et al., 2022). The invasive sporozoites in the tick's salivary glands are transmitted during a blood meal, to a new vertebrate host (Jalovecka et al., 2018).

In vertebrate hosts, the parasite multiplies asexually through invading erythrocytes. Merozoites can re-infect other RBCs in the host after they are liberated from these RBCs. A portion of these parasites develop into gametocytes, both male and female. The sexual phase of the infection starts in the tick's body, when it consumes vertebrate host blood that contains these gametocytes through zygote development. The formed zygote in the tick's midgut goes through sporogony, a process that produces sporozoites (Jalovecka et al., 2018). Asexual replication of *babesia* merozoites within the bovine red blood cells is responsible for parasite pathogenesis (Hakimi et al., 2021), it induces significant intravascular hemolysis hence manifest clinical symptoms (Hunfeld et al., 2008).

Clinical bovine babesiosis is characterized by severe hemolysis of red blood cells, persistent fever, anemia, and frequently hemoglobinuria, which gives the disease's urine a reddish-brown hue and gives it the term "red water." Agalactia, or total milk loss in dairy cows, is an early warning sign (Githaka et al., 2022). Additional symptoms include temporary reduced fertility in bulls and abortion in pregnant cows. When the clinical indications are less severe, jaundice may occasionally be visible, and infected animals with *B. bigemina* frequently have hemoglobinemia (Shkap et al., 2007).

Infection by *B. bovis* are frequently acute or subacute, have a shorter course, and cause more severe symptoms quickly, either resulting in death in fatal instances or a longer recovery period in non-fatal cases (Githaka et al., 2022). Infrequently *B. bovis* can produce additional symptoms by altering erythrocytes, which accumulate in capillaries, particularly in the brain. This condition is known as cerebral babesiosis, and symptoms include hyperesthesia, nystagmus, circling, head pushing, aggressiveness, convulsions, and paralysis (De Vos et al., 2004).

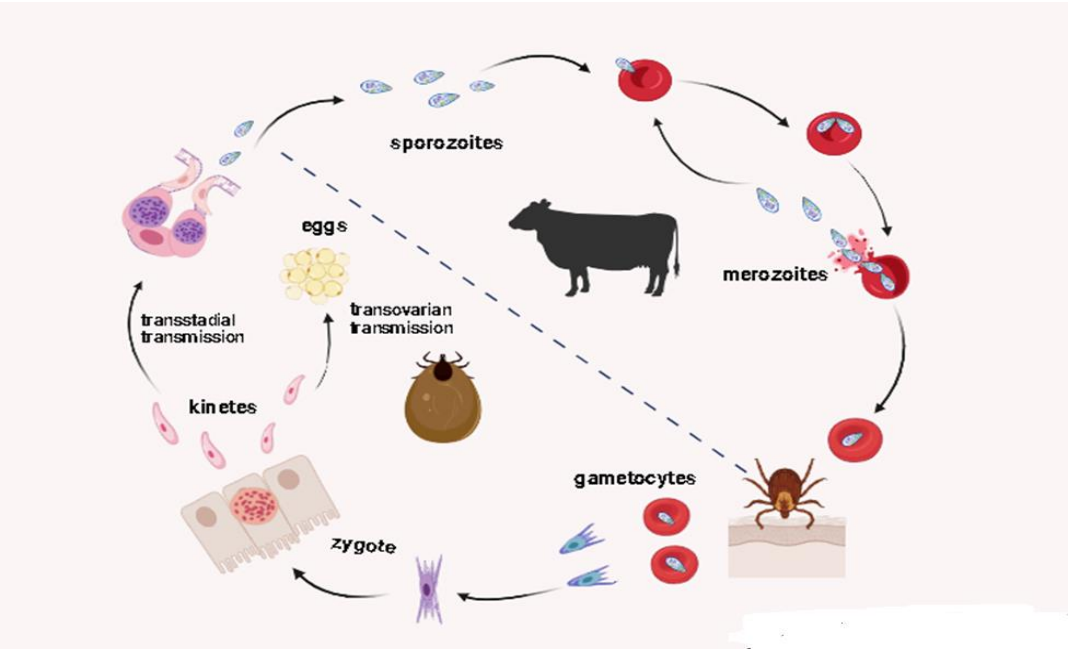

Fig. 1: Life cycle of *Babesia* spp. in bovine

Babesiosis causes high rates of morbidity and mortality in susceptible animals, particularly in exotic and cross-bred cattle. The mortality rates of 30% for *B. bigemina* infection, and 70–80% for *B. bovis* infection have been reported, and are strongly affected by a variety of factors (Githaka et al., 2022) including: previous exposure to *Babesia* species/strain, animal breed, age, sex, flock size, current applied treatments, vaccination status, feed density, seasonal management, tick infestations, domestic pet availability, and management of grazing area (Simking et al., 2014).

Owing to the persistent and hazardous of chemical residues in the environment (Wolstenholme et al., 2004). Development of resistance in ticks as a result of repeated usage of acaricide, as well as the associated costs has restricted acaricide use as a preventative measure (Palmer and McElwain, 1995). Conversely, vaccinations are safe, don't leave any chemical residues (hence don't require withholding periods in animals), are friendly to the environment, and are well-liked by consumers (Dalton and Mulcahy, 2001).

An Overview of Immune System

The immune system is a multifaceted, cohesive network of tissues, organs, and cells that plays specific roles in protecting the body against foreign chemicals and harmful microbes (Nye, 2004).

In mammalian vertebrates, there are two forms of immunity: innate and acquired. The innate immunity is the initial line of defense for a host against infections, which is mediated by phagocytes such as dendritic cells and macrophages. Acquired immunity involve the development of immunological memory and the removal of pathogens during the latter stages of infection (Akira et al., 2006).

Various anatomical barriers to infection together make up the innate immune system, such as physical barriers (the skin), chemical barriers (the acidity of stomach secretions), and biological barriers (the normal microflora of the gastrointestinal tract) (Nye, 2004). Furthermore, phagocytic cells and soluble factors are components of the innate immune system. Soluble factors comprise the acute-phase proteins, messenger proteins known as cytokines, and the complement system (Delves and Roitt, 2004).

The complement system is avital elements of innate immunity, which is made up of a biochemical network of over 30 proteins in plasma and on cellular surfaces. By encouraging phagocytosis or causing direct lysis (cell rupture), the complement system triggers reactions that destroy invasive infections, the complement proteins also control inflammatory responses (Dunkelberger and Song, 2010).

The immune response is regulated by chemical messengers called cytokines (Delves and Roitt, 2000). They are important for phagocytic cell induction into specific infection sites. The primary immune cells engaged in the phagocytosis process are neutrophils, monocytes, and macrophages, these cells engulf and break down invasive pathogens (Iwasaki and Medzhito, 2010).

The recognition of microorganisms done via a variety of germline-encoded pattern-recognition receptors (PRRs). These PRRs share characters: Initially, recognition of microbial components by PRRs identified as pathogen-associated molecular patterns (PAMPs) that are important for the survival of the microorganism. Second, constitutive expression of PRRs in the host and pathogens detection at any stage of their life cycle. Third, PRRs are nonclonal, expressed on all cells of a certain kind, germline-encoded, and immune memory-independent (Akira et al., 2006).

A second line of protection against infections is acquired or adaptive immunity, which takes several days or weeks to fully develop. Since adaptive immunity involves both immunologic "memory" and antigen-specific responses, adaptive immunity is far more sophisticated than innate immunity. The development of immune cells that target and destroy an

invasive pathogen is stimulated by exposure to a specific antigen on the pathogen (Nye, 2004). Immunologic "memory" refers to the fact that antigens are "remembered" and thus, immune responses are more powerful and rapid upon re-exposure to the same infection (Parkin and Cohen, 2001).

The main mediators for the adaptive immune response are B lymphocytes (B cells), and T lymphocytes (T cells). Antibodies are specialized proteins produced by B cells that attach to foreign proteins or pathogens and recognize them in order to destroy them or mark them for destruction by macrophages. The response mediated by antibodies is refers to humoral immunity. In contrast, T cells-lymphocytes that grow in the thymus are responsible for cell-mediated immunity. Various T cell subgroups play distinct functions in adaptive immunity. For instance, killer T cells, or cytotoxic T cells, target and eliminate infected cells directly, whereas helper T cells boost immune responses and support the activity of other lymphocytes (Parkin and Cohen, 2001). Regulatory T cells, also known as suppressor T cells, reduce the activation of the immune responses (Parham, 2005).

Apart from its essential function in innate immunity, the complement system also regulates adaptive immunological responses. It serves as an illustration of how the innate and adaptive immune systems interact (Kohl, 2006; Dunkelberger and Song, 2010). It is commonly known that the Th1/Th2 balance plays a crucial role in the fate of parasites. Th1 responses are linked to the removal of protozoan parasites, while Th2 responses are linked to the uncontrolled proliferation of parasites (Akira et al., 2006). The acquired or adaptive immunity include both passive and active immunity which obtained either naturally or artificially.

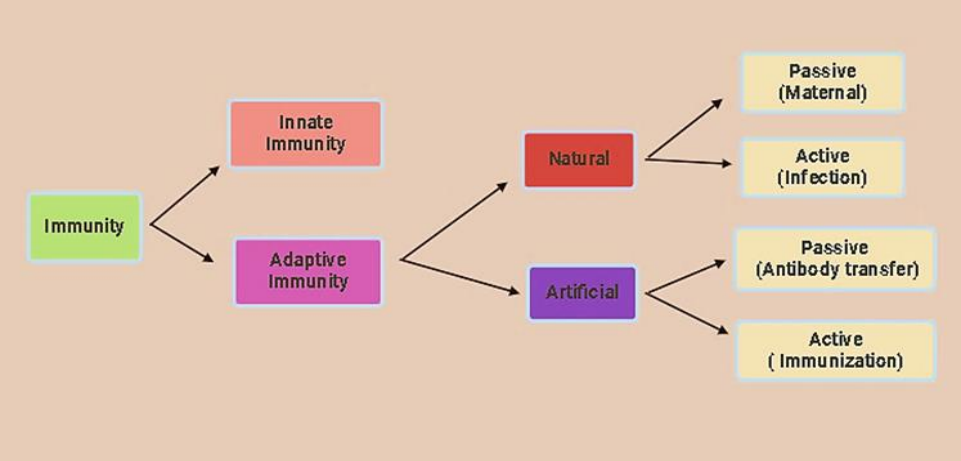

Fig. 2: Diagram of Immune response

Passive immunity is obtained through transferring of pre-made antibodies from one individual to another. Passive immunity can develop spontaneously when maternal antibodies are passed from the mother to the fetus or artificially, when high concentrations of particular antibodies against a virus or toxin are given to non-immune individuals. Immunization with passive components is used to lessen the symptoms of chronic or immunosuppressive conditions, or when there is a significant risk of infection and not enough time for the body to mount an effective defense (Hunt, 2015). Although passive immunity offers instant protection, because the body does not form memories, the patient remains vulnerable to future infections by the same pathogen (Janeway et al., 2001).

Active immunity arises when a pathogen activates B and T cells; memory B and T cells then grow; this leads to the development of the primary immune response. These memory cells will "remember" every unique pathogen that an individual encounters during their lifetime and be able to produce a potent secondary reaction in the event that the pathogen is discovered again (Glenny and Südmersen, 1921).

Due to the body's immune system's ability to adapt, this form of immunity is active and adaptive. Both the humoral and cell-mediated immunity components are frequently involved in active immunity. A natural infection leads to the development of active immunity. Immunological memory results from an individual being exposed to a live pathogen and developing a basic immune response. While vaccination, or chemical containing antigen, can produce artificially developed active immunity. A vaccination, instead of causing the disease's symptoms, elicits a primary response against the antigen (Hunt, 2015).

Immunity against Intra-erythrocytic *Babesia* Parasite

Bovine babesiosis is considered as an acute hazardous disease, when naive animals that are older than a year contract (He et al., 2021). Cattle's immunological response to *B. bovis* infection is crucial to the disease's course, the degree of parasitemia, the intensity of clinical symptoms, and the emergence of immunity (Santos et al., 2023).

In comparison to adults, younger animals exhibit greater resistance, which is spleen-dependent and linked to a robust innate immunity (Hakimi and Verocai, 2023). Additionally, calves have a certain level of immunity due to age-related characteristics that lasts for six to eight months, although adults are more susceptible to the risk of clinical disease (Santos et al., 2023). Concomitant immunity is the term used to describe the resistance that persistently infected cattle typically exhibit against reinfection with similar parasite strains (Brown et al., 2006a).

Both an innate and an adaptive immune response are necessary for defense against intra-erythrocytic protozoan parasites of the genus *Babesia* (Ortiz et al., 2019). The adaptive immune response comprises two aspects: the generation of neutralizing antibodies, and the appearance of parasite antigens to CD4+ T cells by specialized antigen-presenting cells (Montenegro et al., 2022).

The mechanism of protection has been reviewed and illustrated in different ways; a model represented that immunologically naive animals must have a sufficiently robust innate immune response to recover from an acute infection with virulent *B. bovis* parasites. This response triggers the activation of macrophages through IFN-γ, and parasite-derived products which kills the organisms through phagocytosis and produces toxic macrophage metabolites, such as Nitric oxide (NO) (Estes and Brown, 2002). When IFN-γ coexisted with *B. bovis* merozoites or infected erythrocytes, NO generation was triggered (Goff et al., 2002).

Antigen-specific CD4+ T cells play a crucial role in the adaptive immune response by producing IFN-γ in animals that are both well vaccinated and continuously infected yet have controlled parasitemia. Furthermore, IFN-γ stimulates the generation of the neutralizing IgG2 antibody and activates macrophages for effective organism clearance (Estes and Brown, 2002). In cattle, IgG2 is the most effective opsonizing antibody isotype for targeting extracellular parasites and parasite antigens that are visible on the surface of erythrocytes (Ortiz et al., 2019), that avert cattle from being challenged by homologous strains passively (Mahoney, 1986).

Other model has demonstrated that macrophages and NK cells are necessary for recovery from initial infection opposed to CD4+ T cells and antibodies (Aguilar-Delfin et al., 2003). To trigger this kind of innate reaction, the cytokines IL-12 and IFN-γ had to be produced (Brown et al., 2006a). An analysis of cytokine responses during infection represented that recovery from infection depends on early production of IFN-γ and IL- subsequently formation of IgG linked to an IL-4 and IL-10 response (Chen et al., 2000).

In response to *B. bovis*, activated macrophages produce inflammatory cytokines that are crucial for initiating both the innate and acquired immune responses consisting of IL-12, TNF-α, and IL-18. IL-12 stimulated the synthesis of IFN-γ by differentiated Th1 cells and boosted the production of IFN-γ by natural killer (NK) cells, which in turn produced higher amounts of IFN-γ (Brown et al., 1996). TNF-α and IFN-γ work together to stimulate macrophages' production of NO (Goff et al., 1998). Moreover, IL-18 and IL-12 work together to enhance the synthesis of IFN-γ (Shoda et al., 1999).

In cattle younger than six months old, there was an age-related resistance to Babesiosis; these animals are resistant to clinical illness after *B. bovis* exposure. The age-related resistance is rather paradoxical, as infants' innate immune systems are not as established as adults' (Petty and Hunt, 1998). Additional probable explanations for improved resistance of young animals may be related to the abundance of Gamma delta (γδ) T cells, which account for more than 70% of circulating T lymphocytes in ruminants (Hein and Mackay, 1991), or as a result of a diminished pro-inflammatory response for the disease's pathophysiology (Clark and Jacobsen, 1998). This age-related immunity also has a cellular component (Montealegre et al., 1985) in spite of a soluble babesiacidal component (Levy et al., 1982).

Mononuclear phagocytes (MP) are involved as a key effector cell for both innate and primary immune responses and Nitric oxide has been discovered as one of the babesiacidal molecules produced by activated mononuclear phagocytes (Goff et al.,1996). It has been suggested that because NO has a very short half-life, its microbicidal actions are localized to lymphoid organs like the spleen rather than being systemic (Jacobs et al., 1995). Spleen plays a major role in an infection management, since splenomegaly develops during acute babesiosis and splenectomized adult and young animals have significantly greater levels of parasitemia (Goff et al., 2001). The splenic red pulp has the potential to have microbicidal effects because it is sufficiently restricted to allow for NO activity. Furthermore, blood would be expected to show the residual babesiacidal effects of NO (Goff et al., 1998).

The complicated immunological and inflammatory environment in which mononuclear phagocytes operate often determines the MP's functional response, which is influenced by the first regulatory cytokine that the MPs encounter (Erwig et al., 1998). In response to *B. bovis* infection, young calves' protective innate immune response shows type-1 characteristics. This includes early induction of splenic IL-12 and IFN-γ mRNA production, which is followed by a brief period of inducible nitric oxide synthase (iNOS) expression. Conversely, in the spleens of adults who died from the infection, IL-12 and IFN-γ messages appeared later and there was no iNOS present. Furthermore, compared to calves, adults' spleens exhibited larger levels of IL-10 message induction and sustained expression longer. Finally, relative splenic transforming growth factor (TGF)-b mRNA expression levels, and kinetics differed between calves and adults, suggesting a dual regulatory role for this cytokine (Goff et al., 2001).

Numerous factors, such as developmental stage, nutritional stress, and concurrent infection, may affect a cattle's susceptibility to primary *Babesia* infections and possibly of protective immunity formation (Bock et al., 1997). The establishment of protective immunity is also influenced by the host's genotypes for example, purebred *Bos taurus* cattle are more susceptible to *B. bovis* infection than cross-bred or *Bos indicus* cattle (Bock, 1999).

Vaccine and Vaccination

The word "vaccine," comes from the Latin word "vacca," which means cow, was first used by Edward Jenner to refer to the process of vaccinating humans against the related human smallpox virus by inoculating them with the cow pox virus. This illustrates the close relationship between the sciences of infectious diseases in humans and animals (Meeusen et al., 2007).

Vaccination aims to imitate the development of naturally acquired immunity by inoculating immunogenic

components of the pathogen or closely related organisms. The vaccinations may be used to help control, or completely eradicate an infection at the population level, or they may be used to avoid clinical indications of illness following infection (Meeusen et al., 2007).

Vaccinology has emerged as a recognized scientific field that integrates immunology, microbiology, protein chemistry, and molecular biology with business-related concerns such as production costs, regulatory compliance, and profit margins. Developing a vaccine that will defense people and animals from illness is the ultimate goal of each new vaccination (Kahn, 2006).

Substantially the majority of vaccinations continue to use live, attenuated pathogen strains, even though commercial companies typically do not want this kind of immunization because it exposes them to mitigating risks. Additionally, the short shelf life and strain/region specificity of many vaccinations result in production losses. Meanwhile, the live organisms offer greater protection than many subunit vaccines (Lambert et al., 2005).

To increase the efficacy of killed or subunit vaccinations, a deeper comprehension of the immunological and molecular disease processes is probably needed. It is well known that the immune system uses a variety of effector pathways to fight different infections according to the microenvironments and life cycles of each pathogen. The majority of killed and subunit vaccines still primarily depend on the production of neutralizing antibodies (Lambert et al., 2005).

An important development in immunology is the increasing recognition of the pivotal function that innate immunity performs in the functioning of vaccination adjuvants that will influence a frequently overlooked aspect of vaccine development (Pulendran and Ahmed, 2006). Innate immune receptors that have recently been identified are being searched for novel adjuvant chemicals that are active (Pashine et al., 2005). The ligands linked with these receptors, known as pathogen-related molecular patterns, are then employed to either enhance or decrease vaccine responses (Huleatt et al., 2007). When compared to immunizations for humans, the use of adjuvants in veterinary medicine is significantly less limited. Currently, veterinary vaccines that are permitted for use include a wide variety of adjuvant kinds and formulations, while human vaccine use limited to only three adjuvants (Pashine et al., 2005).

Vaccine in Veterinary use

The main objectives of veterinary vaccinations are to enhance companion animal health and wellbeing, boost livestock productivity in an economical way, and stop the spread of diseases from domestic animals and wildlife to humans. Due to these varied goals, the creation of veterinary vaccines has been approached differently, these approaches range from simple yet efficient whole-pathogen preparations to molecularly specified subunit vaccines, or genetically altered organisms, vectored antigen formulations, and naked DNA injections. The creation of a product that will be sold or utilized in the field to accomplish desired results is the ultimate effective result of vaccine research and development (Meeusen et al., 2007).

Mahoney (1967) proved that splenectomized calves infected with *B. bovis* did not exhibit clinical illness after receiving serum from infected donors. The level of protection matched that of cattle recovering from a natural illness, and it was determined that humoral components played a significant role in the effector mechanism that destroyed *B. bovis* in the immune animal. Ultimately, the study results showed that antiserum was highly protective against several infections in donors and that the protective effects were strain-specific (Mahoney, 1979).

Hyperimmune serum appears to be mediated by antibodies in protecting splenectomized calves. It is possible that the merozoites were eliminated upon their emergence from erythrocytes, while the organisms present in the red blood cells in circulation remained unaffected by antibodies. Despite the fact that the initial rate of elimination was higher than the rate of multiplication, indicating that the infected erythrocytes were similarly susceptible to antibody attack, this trait seems to support the concept proposed by Mahoney in 1972 (Mahoney, 1979).

Curnow (1968, 1973) discovered that antibodies against the varied agglutinogens on the surface of infected erythrocytes directly provided protection. To ensure a successful passive transfer, the serum should have antibodies against every type of antigen that the parasite's homologue strain was able to create (Mahoney, 1979).

Live attenuated *Babesia* parasite vaccinations have been used extensively since the mid-1960s to prevent the disease in calves. These vaccinations are widely used in Australia, Argentina, Israel, and South Africa as a preventative measure against bovine babesiosis since they provide a high level of protection (Florin-Christensen et al., 2014).

Relapse of parasitaemia is a characteristic of the hyperimmune serum passive transfer trials. This can happen if the administration of the serum is postponed until the parasitaemia level reaches 103-104/mm^3, or given in modest dose (24 ml/kg) when the level of parasitaemia is between 1 and 102/mm^3. Relapses appeared to be caused by the quantitative relationship between parameters such as the initial antibody concentration and its rate of loss due to antigen-antibody responses, the number of parasites in the blood at the time of injection, and normal catabolism (Mahoney, 1979).

Global climate change-induced increases in animal movement and wildlife-human interactions will necessitate ongoing monitoring for disease outbreaks across the globe, as domestic, farm, and wild animals serve as major reservoirs for a variety of vector-borne human illnesses (Hayes and Gubler, 2006).

Veterinary vaccines have already a significant impact on public health as well as the health, welfare, and productivity of animals, vaccines have recently been used in animal reproduction and industrial procedures (Kahn, 2006). They are meant to increase overall productivity in livestock animals, they lessen the usage of veterinary medications and hormones and the residues of these substances in the human food chain (Meeusen et al., 2007). In order to stay ahead of the

constant threat of newly emerging diseases, there must be constant communication between scientists, animal and human disease control authorities (Kahn, 2006).

The process of generating veterinary vaccines comparing to that of developing human vaccines, has benefits and drawbacks. Considering that animal vaccines have smaller markets and lower sales prices than those for humans, compared to human vaccinations, the possible profits for animal vaccine manufacturers are far smaller. Consequently, despite the complexity and diversity of hosts and pathogens are higher for animal vaccines than for human vaccines, a lot less money is spent on research and development for them. However, the requirements for preclinical trials and regulations, which can account for the majority of the costs associated with developing a human vaccine, are typically less onerous in terms of creating veterinary vaccinations. Moreover, a faster time to market launch and a return on investment in research and development. Veterinary experts have a clear advantage over human vaccine developers in that they can quickly undertake research in the relevant target species (Meeusen et al., 2007).

Although only about 23% of the global market for animal health products is made up of veterinary vaccines, the industry has been growing steadily due to new technological advancements in vaccine production as well as the emergence of new diseases and the ongoing development of drug resistance by pathogens (Meeusen et al., 2007).

Vaccine trials against Bovine Babesiosis

Various procedures have applied for controlling of babesiosis in cattle, although innovation in vaccine preparation make them back word drawing in some procedures that previously applied for immunization against *Babesia* parasite in bovine. In order to combat *B. bovis*, several vaccine approaches were developed, including live attenuated and viral vector vaccines. These strategies used *B. bovis* proteins or whole live parasites, with the latter offering the best protection against babesiosis in cows (Santos et al., 2023).

The live vaccines provide a highly protective effect against babesiosis within a single dose production (de Waal and Combrick, 2006). The immunity is long lasting for at least four years if *B. bovis* vaccine is used, and may be last for less time in case of *B. bigemina* (De Vos and Bock, 2000). It has been documented to endure even after *Babesia* infections are eliminated. Study on drug cured cattle also suggested that the degree of acquired immunity is related to the degree of antigenic stimulation (duration of prior infection) rather than the presence of live parasite (Dalgliesh, 1993). Due to the vaccine's short shelf life, distribution and post-production testing for contamination and efficacy are not feasible in certain nations (De Vos and Jorgensen, 1992).

Despite the fact that there are several essential inadequacies such as the high production costs, the logistics of distribution, and the possibility of pathogen contamination during manufacture (de Waal et al., 2006). In addition, potential for co-infection with contaminating species, especially viruses; persistence of infection in the field; reversion to virulence, temperature liability; storage and transportation a short shelf life of four to seven days at 4˚C, are other drawbacks of receiving a live vaccine (Maqbool et al., 2018).

Developing a vaccine against *B. bovis* using in vitro *Babesia* parasites is known as the "killed vaccine" approach (Florin-Christensen et al., 2014). Which consist of adjuvant and antigens extracted from infected calves' blood or cultured material (Maqbool et al., 2018). The vaccines created using in vitro methods allow for more controlled and consistent environments and have a lower risk of spreading infections (Shkap et al., 2007). However, the duration and the degree of immunity against heterologous challenge lacks sufficient documentation (Maqbool et al., 2018), and the culture-derived organisms may loss its virulence and immunogenicity (De Vos, 1978). Another constraint points of this technique are its requirement for continuous supply of erythrocytes and serum from donor animals, as well as enough laboratory supplies and personnel skill (Florin-Christensen et al., 2014).

The development of novel vaccines that could target many stages of the *Babesia* parasite's life cycle will be greatly aided by recently developed genetic editing techniques (Santos et al., 2023). Following the release of the first whole genome of *B. bovis* in 2007, and sequencing of other *Babesia* spp. genomes important insights into the biology of the parasite have been gained (Brayton et al., 2007). The creation of transfection methods for gene modification and functional analysis has enabled these developments and accelerated the hunt for possible vaccines (Suarez et al., 2019).

Another strategy for the management of bovine babesiosis is vaccination with recombinant protein antigens. The expression of the *B. bovis* MSA-1 and MSA-2c antigens on the merozoite surface aids in the parasite's entry into the bovine erythrocyte (Brown et al., 2006b; Florin-Christensen et al., 2014). The 42 kDa membrane glycoprotein is encoded by a single-copy gene with an open reading frame of 961bp and no intron found in the msa-1 gene locus (Hines et al., 1995). A single copy of the msa-2c gene, which lacks an intron, has a coding sequence that is 795 bp long and produces a 30-kDa protein (Florin- Christensen et al., 2002).

The effectiveness of recombinant vaccines has been doubtful predominantly when fusion proteins have been used such as Thioredoxin. Fusion assembly may alter the recombinant protein's quaternary conformational shape, which may impact the antigenic determinants' interaction and, in turn, its ability to stimulate or protect the immune system. When many recombinant proteins are used simultaneously as immunogens, the results may be superior than when a single protein is used (Willadsen, 2008).

Conclusions

Babesiosis as one of protozoal infection has significant effect on livestock industry, through losses and cost of treatment. Prevention and management of babesiosis in bovine required an effective controlling strategies especially in

endemic areas. The natural resistance in some indigenous bovine breeds may reduce the mortality rate. And persistent immunity is a measured aspect for protection of an infected animals against the next attract of babesiosis caused by virulent *Babesia bovis*. Development of anti- babesial drug resistant as well as absence of effective vaccines are the obstacles impairing babesiosis control in cattle. Immunization have been used for protective purpose against losses caused by bovine babesiosis, although various factors might reduce its application. Vaccination as an advanced procedure can be used for protection against bovine babesiosis. Innovation in manipulation techniques for vaccine preparation is continue for targeting the different pathogen stage and assistance in controlling the devastation diseases.

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